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FAX TRANSMITTAL

DATE:	November 27, 2006
TO:	Examiner Gary Counts
FAX PHONE NO.:	571-273-8300
FROM:	William D. Noonan M.D.
RE:	POSITIVE DETECTION LATERAL-FLOW APPARATUS AND METHOD FOR SMALL AND LARGE ANALYTES (CONT)
OUR FILE:	6122-66637-01
YOUR FILE:	10/686,548
NO. OF PAGES	7 (including this cover page)

PLEASE ACKNOWLEDGE RECEIPT BY RETURN FACSIMILE? ☐Yes ☒No

CONFIRMATION TO FOLLOW? ☐Yes ☒No

CONTACT INFO: If you do not receive all pages or if you have problems receiving transmittal, please call us at (503) 595-5300 as soon as possible and ask for Nicole M. Salazar.

MESSAGE: Please see the attached Declaration.

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WDN:wdn 11/27/06 617132
PATENT

Attorney Reference Number 6122-66637-01
Application Number 10/686,548

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Bauer et al.
Application No. 10/686,548
Filed: October 14, 2003
Confirmation No. 3478
For: POSITIVE DETECTION LATERAL-FLOW APPARATUS AND METHOD FOR SMALL AND LARGE ANALYTES
Examiner: Gary W. Counts
Art Unit: 1641
Attorney Reference No. 6122-66637-01

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450 on the date shown below.

Attorney or Agent for Applicant(s)_____

Date Mailed November 26, 2006

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DRAFT!

SECOND DECLARATION OF DR. ROBERT L. BUCK UNDER RULE 132

1. I hold a Ph.D. in Biochemistry and have worked for 23 years in the field of medical diagnostics. I am currently a consultant for Quantrx Biomedical, and a partner at Labfx, a biotechnology company in Portland, Oregon. For many years I have been involved in research and development related to lateral flow test strips.

2. I have previously performed experiments that were reported in a May 15, 2005 Declaration under Rule 132. Those experiments demonstrated that it is not possible to determine from the disclosure of Boehringer et al. (WO 9839657) that a latex conjugate in a labeling zone and an analyte applied to a sample application zone in that reference would separate before reaching the primary capture zone. Since many variables enter into the separation of the conjugate and the analyte, and Boehringer did not control for those variables, that reference can not be said to disclose differential migration of the conjugate and analyte in a manner that the analyte will reach the primary capture zone ahead of the conjugate. My prior declaration demonstrated, for example, that merely altering the distance between the primary capture zone and the conjugate in the labeling zone will cause the conjugate and analyte to reach the primary

capture zone substantially simultaneously. Since Boehringer does not address or even mention such basic variables that are needed to achieve differential migration, it can not be said to disclose or suggest differential migration.

3. I have now obtained some additional data that illustrate that the selection of parameters that achieve differential migration provides a lateral flow competitive assay that has enhanced binding efficiency in the primary capture zone and provides unexpectedly superior test results. The lateral flow assay we constructed is similar to that shown in the figures of pending U.S. Application No. 10/686,548 in which a lateral flow test strip includes (in a direction of distal flow) a sample application zone, a labeling zone that contains a labeled conjugate, a primary capture zone (PCZ) where an analyte and the conjugate compete for binding, and a secondary capture zone (SCZ) that binds conjugate that is not bound at the primary capture zone. If analyte is absent from a sample applied to the test strip, then conjugate can bind at the PCZ and provide a visible signal in the PCZ as compared to a weak or absent signal in the SCZ. When increasing concentrations of analyte are present in the sample, the analyte competes with the conjugate for binding in the PCZ so that the conjugate binds less completely at the PCZ and unbound conjugate moves along the test strip to instead bind to the SCZ. Hence increasing concentrations of analyte in the sample are indicated by progressively stronger signal (such as color intensity) at the SCZ, and lesser signal (such as color intensity) at the PCZ.

4. We have found that an immediate release of the conjugate from the labeling zone allows it to flow in the same liquid wave front with the analyte so that a large bolus of the conjugate arrives at the PCZ, which overwhelms the binding capacity of the PCZ and results in poor binding efficiency of the conjugate at the primary capture zone. Poor binding of the conjugate bolus results in "leak through" of the conjugate to the SCZ, which is very problematic because it provides a false positive result in the assay. For example, even if no analyte is present in the sample, the poor binding of the bolus to the primary capture zone allows an undesired amount of conjugate to reach the SCZ and provide a signal that can incorrectly indicate the presence of analyte in the sample. In addition, the rapid release of conjugate from the labeling zone allows the conjugate to reach the PCZ at about the same time as the free analyte and diminishes the amount of time the analyte has to bind to the PCZ. This reduced time for binding

of the analyte to the PCZ results in less conjugate being displaced from the SCZ, which in turn results in less signal generation in the SCZ. The reduction in displacement interferes with the ability of the assay to provide a progressively more intense signal from the SCZ (as compared to the PCZ) as the concentration of free analyte increases.

5. These principles were illustrated with an amphetamine assay using the following materials:

Latex conjugate dilution buffer (LCDB)
Millipore blocked glass fiber conjugate release pad (MCRP)
Whatman blocked glass fiber conjugate release pad (WCRP)
Cassette housing for lateral flow test strips
Amphetamine (AMP) test strips containing an anti-AMP PCZ, GAM SCZ, sample and absorption pads prelaminated to backing
Amphetamine solutions in PBS with 250 μg/ml blgG
Digital camera and computer

6. The experiments were performed by spraying the test strip with a PCZ line of mouse monoclonal anti-amphetamine antibody at a concentration of 1.0 mg/ml at a rate of 1.0 μl/cm. The SCZ consisted of a line of Goat anti-mouse IgG sprayed at the same concentration and rate as the PCZ. The conjugate was prepared as a mixture of BSA-amphetamine conjugate and mouse IgG covalently attached to the carboxyl-derivative blue latex.

a. MCRP with un-sonicated latex (MCRP-U)

b. WCRP with unsonicated latex (WCRP-U)

The Millipore conjugate release pad (MCRP) had been empirically found to release this conjugate more quickly than the Whatman conjugate release pad (WCRP), hence the Millipore pad was used to demonstrate relatively delayed release of the conjugate from the pad.

7. The following tables illustrate net intensities of color from the blue latex label in the PCZ and SCZ at 6 minutes and 24 hours after the test began. The data illustrate that the delayed release of the conjugate from the Millipore pad as compared to the immediate release from the Whatman pad resulted in a much greater reduction of binding of conjugate at the PCZ as concentrations of free analyte increased. Hence delayed release of the conjugate from the Millipore pad produced a test in which the colored signal from the PCZ is substantially reduced in the presence of free analyte, thereby providing a superior test that is less confusing to the user.

This reduced signal from the PCZ is believed to be a function of the delayed arrival of the conjugate at the PCZ which improves the efficiency of binding of free analyte at the PCZ and increases displacement of conjugate from the PCZ to the SCZ when free analyte is present in the sample.

6 minutes net intensities

AMP conc		MCRP-U		WCRP-U
0	PCZ	24.28		20.25
	SCZ	8.00		7.69
2.5	PCZ	2.54		6.89
	SCZ	8.32		11.72
5	PCZ	0.94		6.53
	SCZ	9.50		16.67

24 hours net intensities

AMP conc		MCRP-U		WCRP-U
0	PCZ	29.71		18.28
	SCZ	15.73		9.98
2.5	PCZ	4.15		9.93
	SCZ	18.70		16.99
5	PCZ	1.66		7.11
	SCZ	13.61		16.65

8. The following table reports the ratio (PCZ/SCZ) of total amounts of conjugate at the PCZ and SCZ at 6 minutes and 24 hours after the test began.

		PCZ/SCZ		
AMP conc	6 min	24 hr	6 min	24 hr
	MCRP-U	MCRP-U	WCRP-U	WCRP-U
0	3.035	1.888748	2.63329	1.831663
2.5	0.305288	0.221925	0.587884	0.584461
5	0.098947	0.121969	0.391722	0.427027

The data in this table shows that delayed release of the conjugate from the pad improves both primary binding and displacement of conjugate from the PCZ by free analyte. In the absence of free analyte (0) at 6 minutes (which would be a time similar to the period of time the test strip would be allowed to develop in clinical or home use), the amount of conjugate in the PCZ as compared to the SCZ (expressed as PCZ/SCZ) was higher for delayed release of conjugate (MCRP) as compared to the non-delayed release of conjugate (WCRP). This strong signal from the PCZ in the absence of free analyte is helpful in signaling a negative test result (which is indicated by a strong signal from the PCZ as compared to the SCZ). As the concentration of free analyte increases, the ratio of PCZ/SCZ is less for the delayed release situation (MCRP) as compared to the immediate release situation (WCRP), as would be desired for a positive test result in which the signal from the SCZ is greater than from the PCZ.

9. The unexpected superiority of the delayed release of conjugate from the pad is further illustrated in the following table, which reports percent displacement of conjugate from the PCZ at 6 minutes and 24 hours, with the delayed (MCRP) and immediate release (WCRP) of conjugate, for amphetamine concentrations of 0, 2.5 and 5 ng/ml.

		%displacement		
AMP conc	6 min	24 hr	6 min	24 hr
	MCRP-U	MCRP-U	WCRP-U	WCRP-U
0	0	0	0	0
2.5	89.94107	88.25014	77.67493	68.09122
5	96.73979	93.54233	85.12425	76.68638

Delayed release of the conjugate from the pad (MCRP) provided higher percentages of displacement of conjugate from the PCZ as compared to immediate release of the conjugate (WCRP) as the concentration of free analyte increased. Greater displacement of conjugate from the PCZ results in a stronger signal from the SCZ, which is more readily discernible as a positive test for the presence of free analyte.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are made punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

By _____
Robert L. Buck, Ph.D.

Date _____